

Peridomiciliary Breeding Sites of Phlebotomine Sand Flies (Diptera: Psychodidae) in an Endemic Area of American Cutaneous Leishmaniasis in Southeastern Brazil

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Abstract. The occurrence of American cutaneous leishmaniasis (ACL) in areas modified by humans indicates that phlebotomine sand fly vectors breed close to human habitations. Potential peridomiciliary breeding sites of phlebotomines were sampled in an area of transmission of *Leishmania (Viannia) braziliensis* in Southeastern Brazil. Three concentric circles rounding houses and domestic animal shelters, with radii of 20, 40, and 60 m, defined the area to be monitored using adult emergence traps. Of the 67 phlebotomines collected, *Lutzomyia intermedia* comprised 71.6%; *Lutzomyia schreiberei*, 20.9%; and *Lutzomyia migonei*, 4.5%. The predominance of *L. intermedia*, the main species suspected of transmitting *L. (V.) braziliensis* in Southeastern Brazil, indicates its participation in the domiciliary transmission of ACL, providing evidence that the domiciliary ACL transmission cycle might be maintained by phlebotomines that breed close to human habitations. This finding might also help in planning measures that would make the peridomiciliary environment less favorable for phlebotomine breeding sites.

INTRODUCTION

American cutaneous leishmaniasis (ACL) was originally a disease found mainly in forest habitats, occurring from the Southern United States to Argentina.¹ Human infection occurs when people enter forest habitats where phlebotomine sand flies and wild mammals maintain the enzootic cycle of several species of *Leishmania*. The devastation of forests has altered the epidemiological characteristics of ACL throughout South and Central America.^{2–5}

In areas of ancient colonization in Southeastern (SE) Brazil, the disease now occurs in peri-urban areas where the forest is not close to residences, affecting adults and children of both sexes. Dogs and horses are frequently found to be infected with *Leishmania (Viannia) braziliensis*,^{6–9} and the association between human and canine cutaneous leishmaniasis has already been shown in residences where people live with infected dogs^{4,10,11}; in these areas, sand flies are found in high densities in and around houses. The most abundant species include *Lutzomyia intermedia*, *Lutzomyia whitmani*, and *Lutzomyia migonei*, which are considered to be the most likely vectors of *L. (V.) braziliensis* in SE Brazil.^{7,12–14} Considering that human habitations are normally situated more than 300 m from forests remnants, it is possible that the insect vectors breed within the field plots of coffee and bananas that commonly surround houses in this area.¹⁵

Various studies on sand fly breeding sites focusing on the worldwide *Leishmania* transmission have been carried out using different techniques in areas with various levels of disturbance by human activity and in forest habitats.^{16–21} However, in the Americas, there have been few studies on peridomiciliary breeding sites; many studies generally involve isolated collections of a few specimens.^{22–25} In Brazil, Casanova²⁶ detected breeding sites of *L. intermedia* and *L. whitmani* in an ACL area, whereas Deane and Deane²⁷ identified various breeding sites of *Lutzomyia longipalpis* in an area of American visceral leishmaniasis (AVL) transmission, by surveying both forested and

peridomiciliary habitats. In AVL areas, *L. longipalpis* is encountered in habitats that are completely urbanized,^{28–31} providing an epidemiological scenario very different from that observed in ACL-endemic areas.

Thus, little is known about the breeding sites of the sand fly vectors of *L. (V.) braziliensis* or other species causing ACL in peridomiciliary habitats. Demonstrating the presence of breeding sites in proximity to houses, together with the presence of infected domestic animals, might suggest a scenario of domiciliary transmission of the disease independent of the sylvatic cycle of the zoonosis. The objective of this study was to investigate the occurrence of sand fly breeding sites by the systematic monitoring of soil around houses and peridomiciliary outbuildings in an ACL-endemic area of SE Brazil.

MATERIALS

We investigated sand fly breeding sites using an adult emergence trap that was conceived and constructed on the basis of the model described by Disney³² (Figure 1). Each trap consisted of a square wooden frame placed over the soil to enclose a surface area of 0.25 m². A metal plate with a central opening to allow the passage of adult insects was installed inside the box held 10 cm above the soil surface by four metal rods. This part was coated using a paintbrush and castor oil (*Ricinus communis*) to retain any sand flies that emerged within the trap. The upper part of the box was covered with fine mesh that prevented the escape of insects that had not yet touched the oiled plate. Finally, a plastic cover of dimensions 2 × 2 m protected the trap from rain.

METHODS

The study was carried out in an ACL-endemic area located in the municipality of Viana (20°22'S; 40°31'W) in the SE Brazilian state of Espírito Santo, with a mean elevation of 28 m above sea level. According to the Köppen-Geiger classification, the region has a tropical monsoon climate³³ with a mean annual temperature of 24°C (range 17–32°C) and an annual precipitation of ~1,200 mm³⁴; the area presents an irregular relief and has been colonized for more than a century. There

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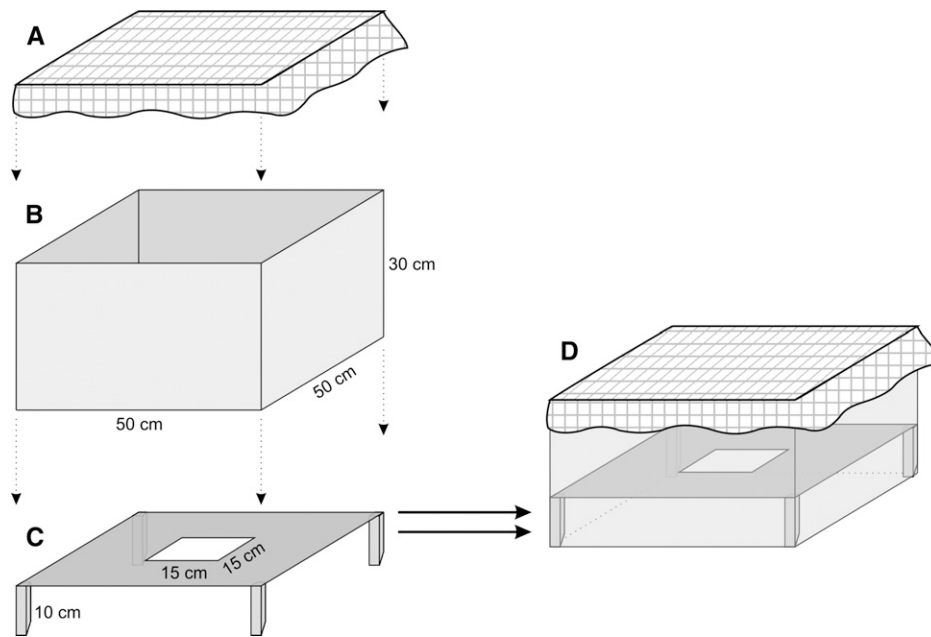


FIGURE 1. (A) Fine mesh, (B) wooden box, (C) metal plate, and (D) assembled trap.

are crops of bananas, coffee, vegetables, and fruit trees as well as remnants of the Atlantic rainforest in the parts unsuitable for agriculture, and generally situated more than 200 m away from residences. The sampled area had two houses occupied by adults and children of both sexes. The peridomicile of these houses included a pigsty, a henhouse, and an outbuilding with four stalls used to store food and agricultural materials and to function as a stable for horses. The domestic animals were principally dogs, pigs, and chickens.

The houses and domestic animal shelters were set very close together, representing the geometric center of the monitored area. Three concentric circles rounding these structures, with radii of 20, 40, and 60 m, defined the area to be monitored. The first sampling range was situated 0–20 m away from the center; the second, 20–40 m; and the third, 40–60 m. Twelve traps (four at each sampling range) were installed simultaneously, allowing for 3 m² of soil to be monitored (1 m² at each sampling range). To avoid accidentally collecting adult sand flies already resting in the soil, each installation was carefully swept before each sampling period.

The traps were installed in patches of shaded soil, rich in organic material and protected from wind and humidity. They were examined every 48 hr to collect sand flies. The traps were moved around weekly within the limits of each station to ensure optimum coverage of patches where sand fly breeding sites could exist.

The study was carried out from May to October 1999, which included a total of 16 weeks of monitoring. Logistic regression analysis was performed to determine whether the presence of sand fly breeding sites in the peridomicile could be predicted according to the distance from houses and domestic animal shelters. Each sampling unit was represented by the four traps installed at each station for 1 week, thereby giving a total of 48 samples (3 stations × 16 weeks).

To compare the population of recently emerged adult sand flies with those active in the area at night, five control collections were performed with two Shannon traps installed in the surrounding dwellings, away from the houses respectively 10

and 50 m from 6:00 to 10:00 PM; the insects were collected off the sides of the trap with a mouth aspirator.³⁵ The collected sand flies were mounted on slides and identified according to the keys of Young and Duncan.³⁶

RESULTS

A total of 1,344 traps were installed, representing a sample effort of 336 m² soil surface during 24 hr. A total of 67 sand flies belonging to 5 species were collected from the 3 stations, which is equivalent to an emergence rate of 19.9 sand flies × 100 m² × day⁻¹. Of these 67 insects, 44 (65.7%) were males and 23 (34.3%) females. Twenty-six (38.8%) were collected at the innermost station; 22 (32.8%), at the second; and 19 (28.4%), at the third (Figure 2).

The most numerous species was *L. intermedia* with 48 individuals (71.6%), followed by *Lutzomyia schreiberi* with 14 (20.9%). *Lutzomyia intermedia* were the most abundant at the first and second stations with 22 and 18 specimens, respectively. *Lutzomyia schreiberi* was the most abundant at the third station where 10 specimens were collected (Figure 2). Logistic regression analysis revealed that the probability of sand fly occurrence did not change with distance from the houses and animal shelters (degrees of freedom [df] = 1, *N* = 48; *L. intermedia*: $\chi^2 = 3.2$, *P* = 0.08; *L. schreiberi*: $\chi^2 = 5.5$, *P* = 0.02; *L. migonei*: $\chi^2 = 0.6$, *P* = 0.46; *Lutzomyia fischeri*: $\chi^2 = 0.0$, *P* = 1.0; *Lutzomyia ferreirana*: $\chi^2 = 0$, *P* = 1.0; total: $\chi^2 = 0.2$, *P* = 0.69).

The control collections included 5,812 sand flies of 8 species, of which 4,915 (84.6%) were males and 897 (15.4%) females. Once again, *L. intermedia* was the most abundant with 5,646 specimens (97.1%), followed by *L. migonei* with 92 (1.6%; Figure 3).

DISCUSSION

Systematic monitoring of the soil surface revealed the existence of suitable sites for oviposition and the development of immature sand flies in the peridomicile.

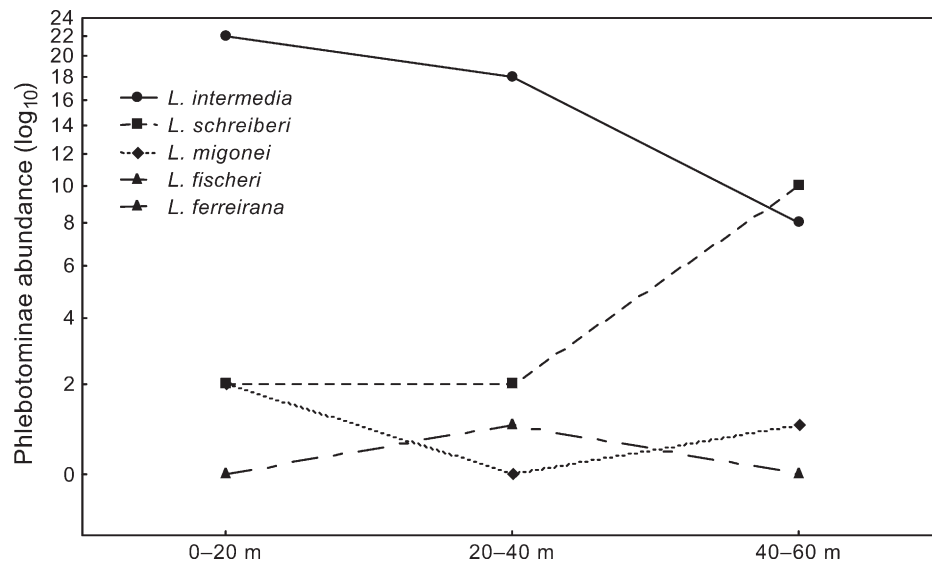


FIGURE 2. Abundance of phlebotomine sand flies collected using soil monitoring traps installed in the peridomicile at three distances from houses in a cutaneous leishmaniasis-endemic area of SE Brazil from May to October 1999.

Natural breeding sites of *L. intermedia* and *L. whitmani* have already been identified in SE Brazil in both sylvatic and peridomiciliary habitats.^{23,24,26} In Northeastern Brazil, Deane and Deane²⁷ found breeding sites of *L. longipalpis* in the vicinity of human habitations, which explains the occurrence of this visceral leishmaniasis vector in ancient colonized areas, including urban ones. In this study, various species of sand flies, principally *L. intermedia* and *L. migonei*, which probably transmit *Leishmania* to humans and dogs, were found to be breeding around human habitations.^{14,37} These species were found to be naturally infected with *L. (V.) braziliensis*, thus providing further evidence of their participation in the epidemiological cycle of ACL.^{38,39}

Several authors hypothesize the occurrence of a domiciliary transmission cycle of ACL involving humans and domestic animals, especially dogs and horses.^{3,4,9,12,40} The discovery of peridomiciliary breeding sites of sand fly vectors of *L. (V.) braziliensis* supports this hypothesis, suggesting that they

are able to survive and transmit *Leishmania* in areas devoid of forests. Even with the number of sand flies collected being relatively low, the results were relevant; since the entire monitored area (covered by the 60 m radius) was sampled, the estimations show the emergence of more than 2,000 phlebotomine sand flies per day. This estimation predicts the maintenance of a wide population in the peridomicile, independent of forests.

The generalist behavior of *L. intermedia* explains its greater occurrence observed in the breeding sites. Its greater ability to reproduce around houses would explain its high density and its predominance over other species in this environment, as observed in the nocturnal collections. The homogenous occurrence of *L. intermedia* in samples at three different distance stations from houses and domestic animal shelters is concordant with the findings of studies that identified sand fly breeding sites in a variety of ecotopes.^{21,25,27} On the other hand, the present results contradict those of other studies

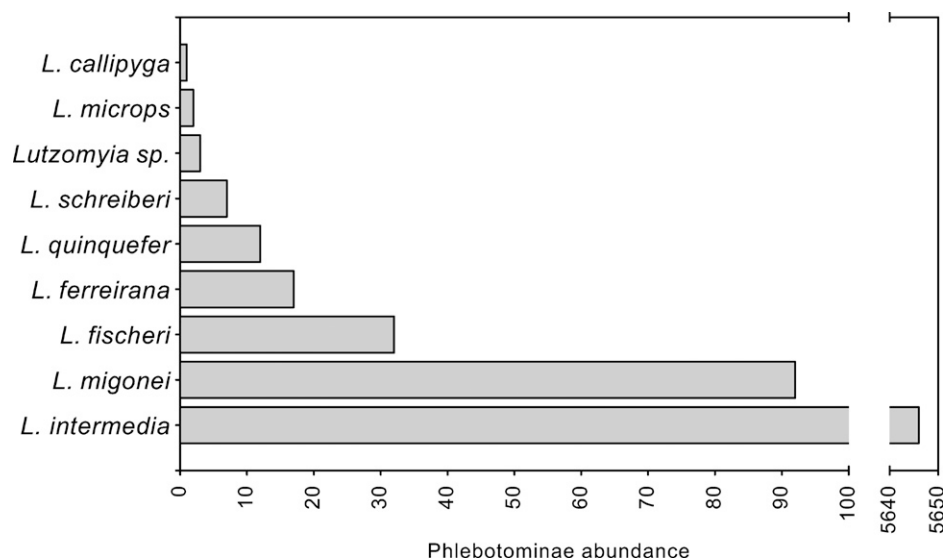


FIGURE 3. Phlebotomine sand flies collected in Shannon traps installed from 6:00 to 10:00 PM in the peridomiciles of houses where breeding sites were identified in a cutaneous leishmaniasis-endemic area of SE Brazil, May–September 1999.

that indicate a preference for breeding sites close to domestic animals, where adults and immatures would have greater access to food and shelter.^{21,23,24} Previous experiments show that female *L. longipalpis* searching for breeding sites are attracted by volatile compounds present in vertebrate feces and by pheromones secreted by other females onto eggs during oviposition.^{41,42}

The presence of *L. schreiberi*, *L. migonei*, *L. fischeri*, and *L. ferreirana* in peridomestic breeding sites, even in small numbers, shows that these species are also able to survive in habitats modified by humans. In addition to *L. intermedia*, *L. migonei* may also be involved in the domiciliary transmission of ACL.^{14,37} Both *L. fischeri* and *L. ferreirana* were recently found to be infected with *Leishmania* (*Viannia*) in rural areas of SE and S Brazil where ACL is endemic.^{43,44}

Knowledge of the peridomestic breeding sites of phlebotomine sand flies could help orient environmental intervention measures that would impede the breeding of these insects and justify the application of insecticides to control household transmission.⁴⁵ It was found that reorganizing and cleaning of the peridomestic and the application of residual insecticides in houses and outbuildings significantly reduces sand fly populations in ACL-endemic areas modified by human activities.^{46–49} We suggest that similar studies in areas with urban transmission of AVL be conducted, because identifying the breeding sites of *L. longipalpis* could enhance the planning of vector control measures.

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